PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Gary Ruvkun et al.	Art Unit:	1633
Serial No.:	09/205,658	Examiner:	Sumesh Kaushal, Ph.D.
Filed:	December 3, 1998		
Title:	THERAPEUTIC AND DIAGNOSTIC TOOLS FOR IMPAIRED GLUCOSE TOLERANCE CONDITIONS		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF GARY RUVKUN, PH.D.

I declare:

1. I am an inventor on the above-captioned patent application.

2. I have read the Office Action mailed on January 10, 2000 and the Advisory Action mailed on July 27, 2000.

3. It is my opinion that a person of ordinary skill in the field of *C. elegans* genetics could have broadly practiced the invention as claimed by using the teachings in the patent application in combination with the knowledge and techniques known in the

field at the time the application was filed.

4. To directly demonstrate the functional similarity between the nematode *daf-18* and the mammalian PTEN genes, my laboratory has constructed transgenic nematodes and has demonstrated that expression of a human PTEN gene rescues *C. elegans daf-18* mutants. The experiment was performed by constructing transgenic *C. elegans* expressing *daf-18* and PTEN cDNAs, and assessing the ability of these genes to revert the phenotype of *daf-2*; *daf-18* double mutant *C. elegans* to the phenotype of a *daf-2* mutant *C. elegans*, thereby indicating successful rescue of the *daf-18* mutation.

5. Specifically, the *daf-18* rescue experiment was performed as follows. *daf-18* and PTEN "minigenes" were constructed using cDNAs and native *daf-18* 5' flanking sequence (approximately 1.0 kb) and 3' flanking sequence (approximately 2.4 kb). These minigenes were constructed by standard PCR overlap extension techniques using nested primers. The primers and strategy used for the PCR overlap extension techniques are shown in attached Figure 1. Primers to the 5' and 3' flanking regions were designed based on the genomic sequence, and were empirically tested using standard methods. Restriction enzyme digests confirmed the identity of the final PCR products.

6. Standard techniques referred to in our specification at page 38 were used to generate the transgenic *C. elegans* (Mello *et al.*, EMBO J. 10:3959-70, 1991). Specifically, the minigenes (at a concentration of 9 ng/ml) were co-injected with a plasmid encoding green fluorescent protein (GFP) under the control of the *sur-5*

promoter (*sur-5*:GFP) into *daf-2*(*e1370*); *daf-18*(*mg198*) mutant *C. elegans*. *sur-5*:GFP is a widely expressed GFP that serves as a convenient co-injection marker for identification of transgenic *C. elegans*. Double mutant *C. elegans* were chosen to be injected so that rescue of the *Daf-d* phenotype (i.e., no dauer formation at 25°C) of *daf-2*(*e1370*); *daf-18*(*mg198*) mutants could be easily assayed. Minigene rescue of *daf-18* would result in a phenotypic reversion of the injected *daf-2*(*e1370*); *daf-18*(*mg198*) strain to that of a *daf-2*(*e1370*) phenotype, resulting in a high percentage of GFP-expressing *C. elegans* that formed dauers at 25°C, but not at lower temperatures, for example, 20°C. *sur-5*:GFP was injected alone as a negative control, and a *daf-18* rescuing genomic PCR fragment was coinjected with *sur-5*:GFP as a positive control.

7. GFP-expressing F1 *C. elegans* were picked for egg lay at 25°C, and F2 *C. elegans* were then scored for GFP expression and the dauer phenotype. Results of the injections are shown below in Table 1.

Table 1. Human PTEN rescues *daf-18*(*mg198*)

Injected Transgene	# GFP-positive *C. elegans*	# dauers	% rescue
none	53	0	0
daf-18 genomic	16	16	100
daf-18 minigene	17	17	100
PTEN minigene	33	33	100

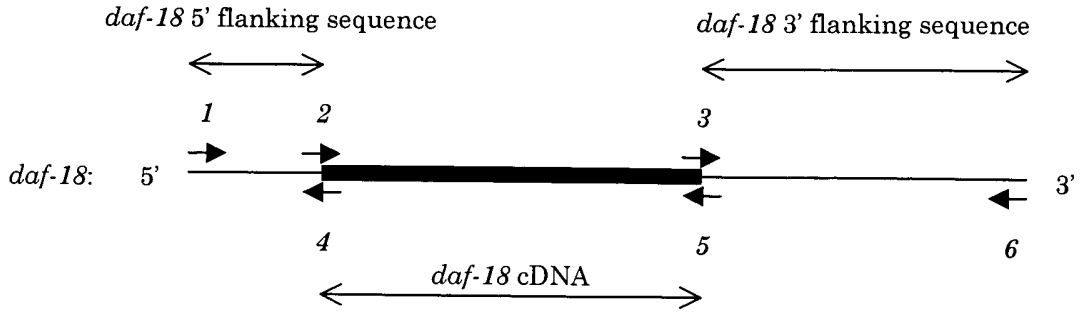
Percent rescue was calculated by dividing the number of dauers by the number of GFP-positive *C. elegans*. These results indicate that both the *daf-18* transgene and the PTEN transgene mediate the rescue of a *daf-18*(*mg198*) mutant at a level of 100%. The negative control resulted in 0% rescue of *daf-18*(*mg198*), while the positive *daf-18* genomic control yielded 100% rescue of *daf-18*(*mg198*). These data demonstrate the functional orthology between DAF-18 and PTEN.

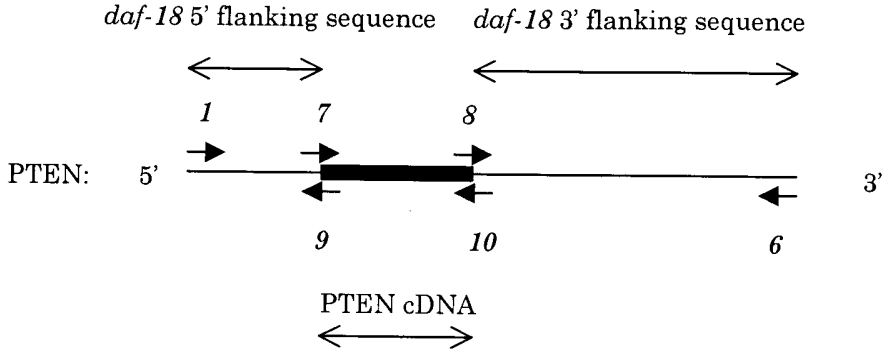
8. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

1/25/01
Date

Gary Ruvkun, Ph.D.

\\Nrserver\documents\00786\351xxx\00786.351004 Declaration of Dr. Ruvkun for CPA.wpd





Oligonucleotide sequences (5' → 3'):

1: CCACGGAAACTCATTTCTG
2: AGGTACATCTACTAACCCCCAATGGTTACTCCTCCTCCAGATGTG
3: TTTGATCAAGCTATTTATTTGTAAACCTAAAACAAAACTTTTAGAAGA
4: CACATCTGGAGGAGGAGTAACCATTGGGGGTTAGTAGATGTACCT
5: TCTTCTAAAAGTTTTGTTTTAGGTTTACAAATAAATAGCTTGATCAAA
6: CGCAATCGCTGCAATATTCGTTGC
7: AGGTACATCTACTAACCCCCAATGACAGCCATCATCAAAGAGATC
8: CATACACAAATTACAAAAGTCTGAACCTAAAACAAAACTTTTAGAAGA
9: GATCTCTTTGATGATGGCTGTCATTGGGGGTTAGTAGATGTACCT
10: TCTTCTAAAAGTTTTGTTTTAGGTTCAGACTTTTGTAATTTGTGTATG

Figure 1